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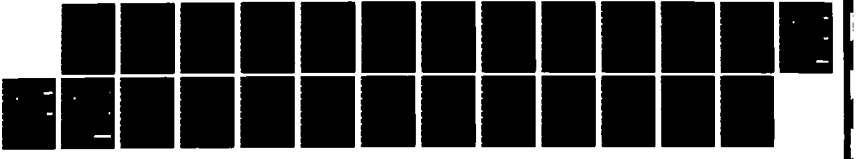

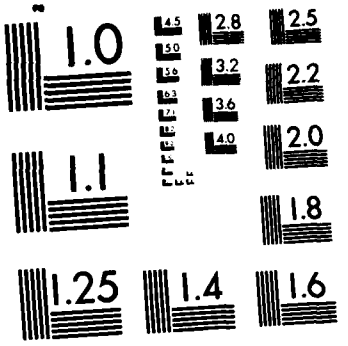

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ANIMAL MODELS FOR THE PREVENTION OF ACUTE AND CHRONIC GRAFT-VS-HOST DISEASE

Annual Summary Report

Michael T. Gallagher, Ph.D. and Lawrence D. Petz, M.D.

September 1982

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These studies considered the effect of monoclonal antibodies to several T-cell differentiation antigens on graft-versus-host disease in a murine model of bone marrow transplantation. The monoclonal antibodies were used for in vitro treatment of bone marrow and/or spleen cells prior to transplantation and were also used to treat established graft-versus-host disease by in vivo injections. The results increase our understanding of transplantation biology and can form the basis for clinical studies.

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SUMMARY

One of the purposes of the work was to cevelop a better understancing of the mechanism whereoy pre-treatment of a mixture of bone marrow and spleen cells (which are used in a murine model of bone marrow transplantation) with rabbit antisera against murine lymph node tissue could effect not only acute graft-versus-host disease mortality but also chronic graft-versus-host disease mortality. The second major objective of the resesarch was to evaluate monoclonal antibodies to several T cell differentiation antigens both as agents for the in vitro pretreatment of bone marrow and soleen cell grafts to modify their graft-versus-host disease potential.and also as agents for the in vivo treatment of estaolisned graft-versus-host disease.

The methods used involved a well characterized murine model of bone marrow transplantation in which recipient mice are treated with lethal doses of total body irradiation and reconstituted with allogeneic bone marrow cells or a combination of allogeneic bone marrow and soleen cells. Monoclonal antibodies against T-cell differentiation antigens were used to treat the in vitro cells prior to transplantation in order to eliminate T-cells. The monoclonal antibodies were also used in in vivo experiments in an effort to influence the course of estaolished graft-versus-host disease.

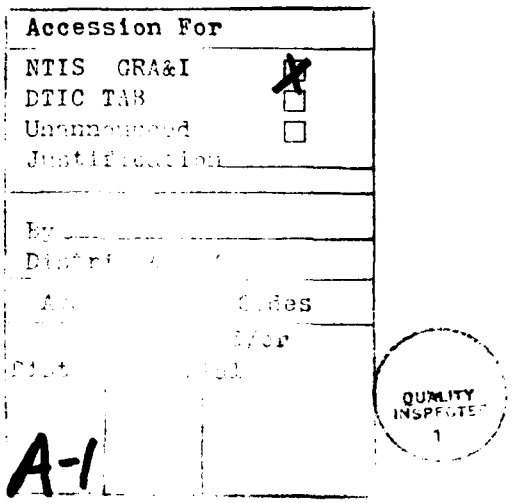
Preliminary studies indicate that the monoclonal antibodies reacted to high titer as measured by cytotoxicity tests. However, higher concentrations were needed in reactions with bone marrow cells in vitro in order to have a consistent innibitory effect on graft-versus-host disease.

Attempts to ameliorate established graft-versus-host disease by in vivo injections of monoclonal antibodies generally had little effect. However, treatment with anti-LYT 1.2 was effective in reducing mortality from 100% to 50% at 21 days and with a prolongation of mean survival time from 10 days to 19.6 days.

Conclusions of the studies thus far are that in vitro treatment of bone marrow cells or bone marrow and soleen cells with low concentrations of monoclonal antibodies generally have no significant effect on mortality or mean survival time in a murine allogeneic bone marrow transplantation model. However, higher concentrations of selected monoclonal antibodies did prolong survival and decrease mortality from graft-versus-host disease. Treating established graft-versus-host disease with in vivo injections of monoclonal antibodies to T-cell differentiation antigens generally had little effect on established acute or chronic graft-versus-host disease, except in one experiment using anti-LYT 1.2.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).



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Over the past 11 months, we have made substantial progress towards realizing the objectives of our contract entitled "Animal Models for the Prevention of Acute and Chronic Graft-Versus-Host Disease". The objectives of our research were two-fold. First, we designed experiments which would allow us to better understand the mechanism(s) whereby a single in vitro pretreatment of a bone marrow and spleen cell graft with rabbit antisera made against murine lymph node tissue could effect not only acute graft-versus-host disease (GVHD) mortality but also late developing chronic GVHD mortality. The understanding of this phenomenon could lead to better methods of prophylaxis and treatment of chronic GVHD in the clinic.

Our second major objective was to evaluate monoclonal antibodies to several T-cell differentiation antigens as potential agents for the in vitro pretreatment of murine bone marrow and spleen cell grafts to modify their GVHD potential.

Both objectives have the same overall goal of increasing the number of patients who could benefit from bone marrow transplantation by elimination or modifying GVHD which is the major obstacle to a more widespread use of allogeneic bone marrow transplantation today. Militarily this would make bone marrow transplantation a more viable therapy in the event of nuclear accidents or limited nuclear war.

I. A Study of the Incidence of Chronic GVHD Mortality in Mice Receiving Mixtures of Bone Marrow and Spleen or Bone Marrow Cells Alone Pretreated with Rabbit Antimouse Antisera

These experiments were designed to evaluate the effect of a single pretreatment with rabbit antimouse lymph node sera (RAMLNS) on the chronic GVHD potential of murine bone marrow grafts. We have previously shown that numerous rabbit antimouse antisera are capable of reducing the acute GVHD potential of mixtures of murine bone marrow and spleen cells. In these experiments, we also reported that mice receiving spleen and bone marrow cells that had been pretreated with rabbit antisera to mouse lymph node cells (RAMLNS and RAMPLNS) showed a greatly reduced incidence of chronic GVHD mortality (Table I). How a single in vitro pretreatment could effect the incidence of chronic GVHD mortality was a puzzle. Chronic GVHD in the murine model is thought to be the result of recognition of host antigens by immune competent cells derived from stem cells in the initial inoculum. How could a single pretreatment effect cells not present at the time of the treatment? Our initial observations were made in mice receiving pretreated mixtures of bone marrow and spleen cells. If the effect of the RAMLNS was due to complete elimination of lymphoid elements (both mature and immature), one could expect chronic GVHD mortality to be the same in mice receiving treated mixtures of bone marrow and spleen cells or treated bone marrow cells alone. However, if alteration of mature T-cell subpopulations in the graft was involved (i.e., killing of helper T-cells while sparing suppressor T-cells), one might expect the antibodies to be effective on mixtures of bone marrow and spleen but not on bone marrow cells alone. These results would be expected because bone marrow and spleen cell mixtures contain large numbers of T-cells (thus, various subpopulations), whereas bone marrow cells alone have almost no mature T-cells.

TABLE I

THE EFFECT OF RABBIT ANTIMOUSE LYMPH NODE SERA ON THE ACUTE AND CHRONIC GVHD POTENTIAL OF MURINE SPLEEN AND BONE MARROW CELLS AT BAYLOR COLLEGE OF MEDICINE

Lethally Irradiated Recipient Strain	Number	Treatment	% Mortality 14 Day (Acute GVHD)	% Mortality 90 Day (Chronic GVHD)
$(C57XA)_{f1}$	18	A BM & SPLEEN	100%	--
$(C57XA)_{f1}$	18	A BM & SPLEEN R_x RAMMLNS	0%	5%
$(C57XA)_{f1}$	18	A BM & SPLEEN R_x RAMPLNS	0%	0%

Initially, we tested the RAMLNS (RAMMLNS AND RAMPLNS) for effectiveness in an acute GVHD model. The results of these experiments are shown in Table II. This table presents the combined results of four separate experiments. Twenty-nine lethally irradiated mice receiving no graft died within 14 days of aplasia. This irradiation control group served to verify that the mice were indeed lethally irradiated. In the cell control group, again, 29 of 29 $(C57XA)_{f1}$ hybrid mice which were lethally irradiated and injected with $5X10^7$ untreated A strain bone marrow and spleen cells died by day 14. This group died of acute GVHD not of aplasia as did the irradiation controls. The third group died of acute GVHD not of aplasia as did the irradiation controls. The third group received mixtures of A strain bone marrow and spleen cells that had been pretreated in vitro with RAMPLNS. In this group, acute GVHD mortality was reduced from 100% to 50%. Finally, group 4 received mixtures of bone marrow and spleen cells pretreated with RAMMLNS. In this group, the acute GVHD mortality was only 20%. The experiments demonstrated that the RAMLNS were still highly effective in abrogation of acute GVHD mortality. The differences in the percent acute GVHD mortality seen in the treated groups in the present experiments and our previously published results (Table I) were not unexpected since the previously reported data were obtained in a barrier sustained specific pathogen free (SPF) mouse colony at Baylor College of Medicine, whereas the data obtained at the City of Hope National Medical Center used commercially obtained SPF mice housed in a clean conventional mouse colony. It is well known that GVHD mortality is complicated by many factors and that mortality is usually higher in conventional colonies.

Having established the basic model and the effectiveness of the RAMLNS in our laboratory, we then proceeded to test the hypothesis outlined above. THe results of several experiments measuring the effects of a single in vitro pretreatment with RAMLNS on the chronic GVHD mortality produced by bone marrow cells alone is presented in Table III. Thirty-six mice receiving lethal irradiation and 10^7 untreated A strain bone marrow cells showed 70% chronic GVHD mortality by day 60. Nineteen $(C57XA)_{f1}$ hybrids receiving lethal irradiation and 10^7 A strain bone marrow cells pretreated in vitro with RAMLNS showed only 16% chronic GVHD mortality in the same time period. In short, a single in vitro pretreatment of bone marrow cells with RAMLNS resulted in a greatly reduced chronic GVHD mortality. This result supports the hypothesis that complete elimination of lymphoid elements (both mature and immature), not selective removal or concentration of a particular mature T-cell subpopulation is the mechanism whereby RAMLNS has it effect of chronic GVHD mortality. We are currently expanding the number of mice in these groups.

II. The Use of Monoclonal Anti-T-Cell Antibodies to Characterize the T-Cell Populations of Mice Receiving Pretreated Murine Cell Grafts

In addition to the experiments reported above, we have also used monoclonal antibodies against various T-cell antigens (THY 1.2, LYT 1.2, LYT 2.2) to characterize the T-cell populations in lymphoid organs of $(C57XA)_{f1}$ hybrid mice receiving A strain bone marrow cells either untreated or pretreated with RAMLNS in vitro. Syngeneically reconstituted mice were used as controls in these experiments. Again, these experiments are directed towards a better understanding of the mechanism(s) by which a single in vitro pretreatment with RAMLNS can effect chronic GVHD mortality. If alteration of T-cell balance is involved (i.e., increased suppressor-decreased

TABLE II

THE EFFECT OF RABBIT ANTIMOUSE LYMPH NODE SERA ON THE ACUTE GVHD POTENTIAL OF MURINE SPLEEN AND BONE MARROW CELLS AT CITY OF HOPE NATIONAL MEDICAL CENTER

Lethally Irradiated Recipient Strain	Number	Treatment	% Mortality 14 Day (Acute GVHD)
$(C57XA)_{f1}$	29	None	100%
$(C57XA)_{f1}$	29	A BM & SPLEEN	100%
$(C57XA)_{f1}$	24	A BM & SPLEEN R_x RAMPLNS	50%
$(C57XA)_{f1}$	24	A BM & SPLEEN R_x RAMMLNS	20%

TABLE III

THE EFFECT OF A SINGLE IN VITRO PRETREATMENT WITH RABBIT ANTIMOUSE LYMPH NODE SERA ON THE CHRONIC GVHD POTENTIAL OF MURINE BONE MARROW CELLS

Lethally Irradiated Recipient Strain	Number	Treatment	% Mortality 60 Day (Chronic GVHD)
$(C57XA)_{f1}$	36	A BM	70%
$(C57XA)_{f1}$	19	A BM R_X RAMLNS	16%

helper), one might expect differences in T-cell subpopulations in mice receiving treated or untreated cells. However, if complete removal of lymphoid elements is the mechanism whereby a single treatment is effective, differences in T-cell subpopulations might not be observed. The results of experiments measuring T-cell subpopulations in normal A strain mice, autologously repopulated A strain mice, $(C57XA)_{f1}$ hybrids repopulated with untreated A strain cells and $(C57XA)_{f1}$ hybrids repopulated with RAMLNS treated A strain cells are presented in Figures 1, 2, and 3. These figures show data from approximately 90 days post-transplant. The data presented here is given in terms of specific cytotoxicity (i.e., the cytotoxicity seen when normal sera and complement are used is subtracted from the cytotoxicity seen when the antibody and complement is used). Figure 1 shows the cytotoxicity of anti-THY 1.2 monoclonal antibody against thymus, spleen, and lymph node cells of the 4 groups of mice under study. All irradiation chimeras have more cells in the thymus which can be killed by anti-THY 1.2 and complement than do the normal controls. This may reflect the increased lymphocyte production needed to repopulate the tissues of lethally irradiated mice. However, there was no significant differences seen in the number of THY 1.2 bearing cells in the allogeneically reconstituted mice receiving untreated marrow (ALLOBM) and the allogeneically reconstituted mice receiving bone marrow pretreated with RAMLNS in vitro ($ALLOBMR_X$).

In the spleen there were no differences seen in the THY 1.2 population in any of the four groups of mice studied Finally, in the lymph node, all irradiation chimeras showed lower numbers of THY 1.2 sensitive cells possibly reflecting incomplete reconstitution of the peripheral lymphoid system. Again, there were no significant differences seen between the ALLOBM mice and the $ALLOBMR_X$ mice. Figure 2 shows the cytotoxicity of anti-LYT 1.2 monoclonal antibody against thymus, spleen and lymph node cells of the four groups of mice. In the thymus, normal and autologously reconstituted mice had undetectable levels of LYT 1.2 bearing cells, while both allogeneically reconstituted groups showed low levels of LYT 1.2 bearing cells (4% and 6.3%). This again did not represent a significant difference in ALLOBM and $ALLOBMR_X$ mice. In the spleen, a small but insignificant difference was observed in that ALLOBM mice had no detectable LYT 1.2 bearing cells whereas $ALLOBMR_X$ mice had 2.5% LYT 1.2 bearing cells. In the lymph nodes, ALLOBM and $ALLOBMR_X$ mice had 6% and 8.8% LYT 1.2 positive cells whereas normal or autologously reconstituted mice had 3.5% and 4%.

Figure 3 shows the cytotoxicity of LYT 2.2 monoclonal antibodies against thymus, spleen and lymph node cells of normal, autologously reconstituted ALLOBM and $ALLOBMR_X$ mice. All irradiation chimeras showed higher than normal levels of LYT 2.2 bearing cells in the thymus, however, there were no significant differences between the ALLOBM and $ALLOBMR_X$ groups. In the spleen, all mice had low levels of detectable LYT 2.2 bearing cells. Finally, in the lymph nodes all mice showed low levels of LYT 2.2 positive cells with the $ALLOBMR_X$ group having the highest value at approximately 5%. THY 1 is a pan T-cell antigen of mice that is present in high concentration on mouse thymocytes and in lower concentration on peripheral T-cells. Most mice have the THY 1.2 allele as do the A strain mice used in our study. The LYT 1 antigen is used to mark the helper T-cells in our study. We use the LYT 2 antigen to mark the suppressor/cytotoxic T-cell compartment. The helper T-cell population in mice is actually marked with both LYT 1 and LYT 2 antigens. The cytotoxic/suppressor T-cell population is marked with LYT 2 and LYT 3 antigens. Thus, LYT 2 antisera should kill helper and cytotoxic/

FIGURE 1. % THY 1.2 BEARING CELLS IN:

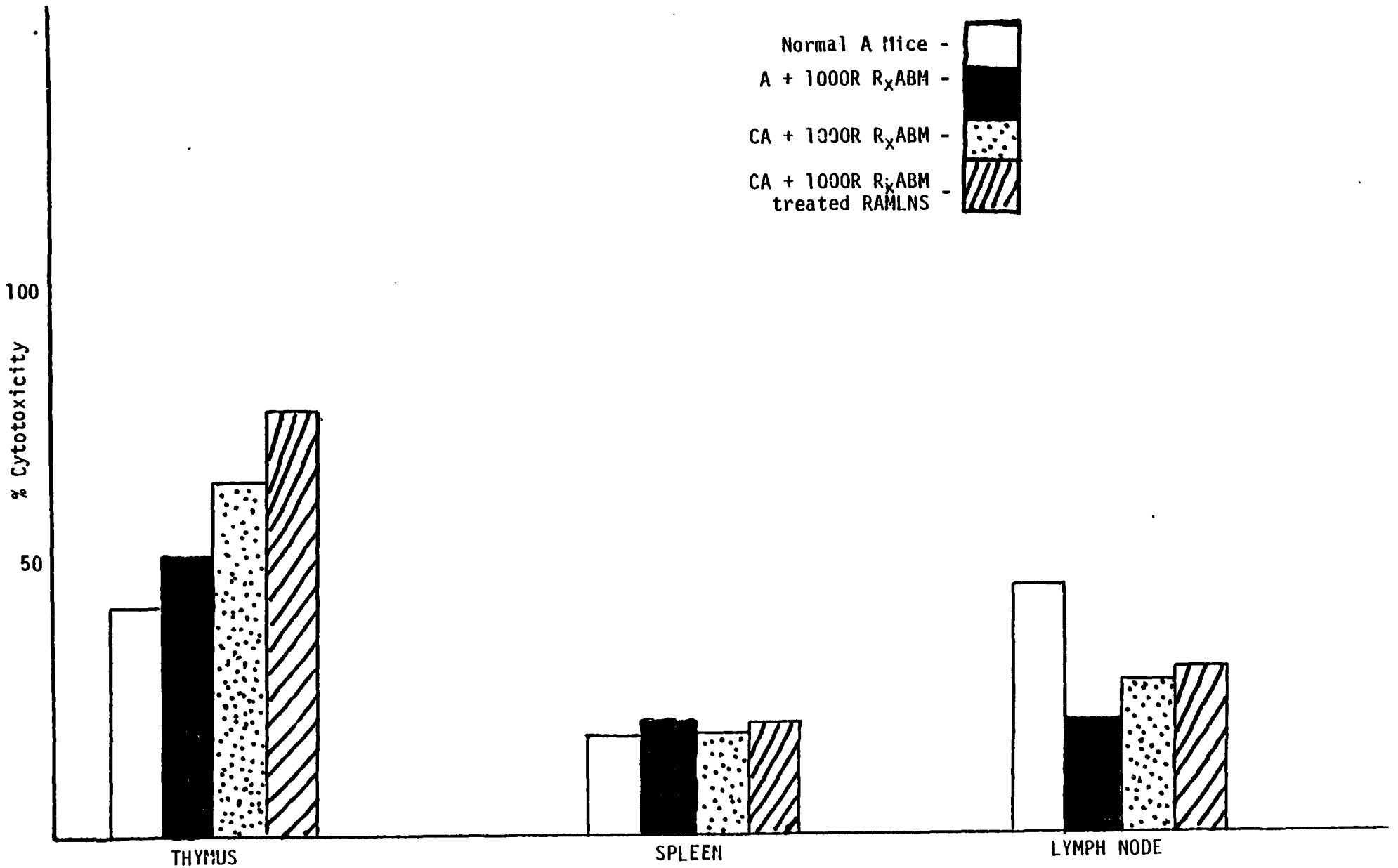


FIGURE 2. % LYT 1.2 BEARING CELLS IN:

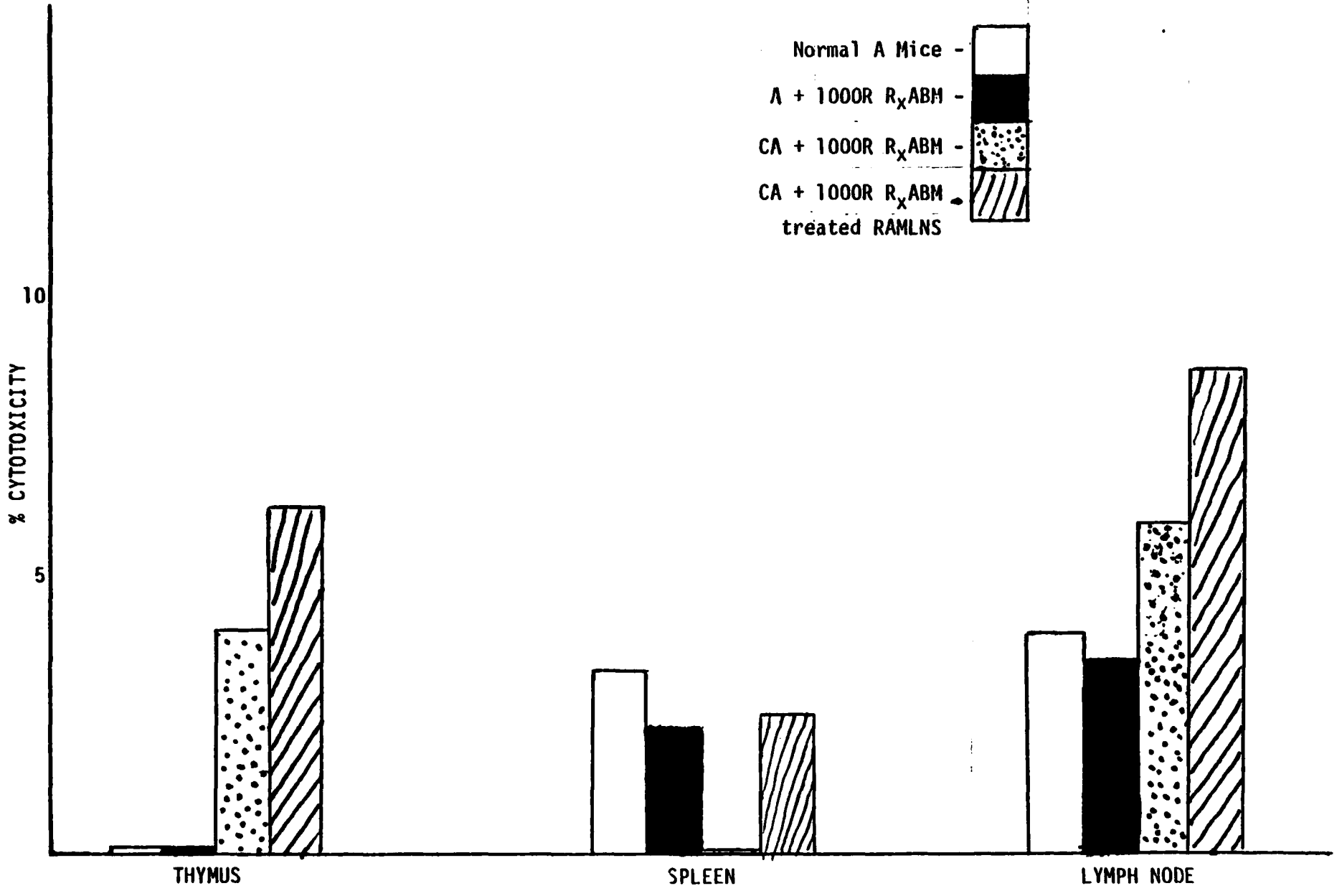
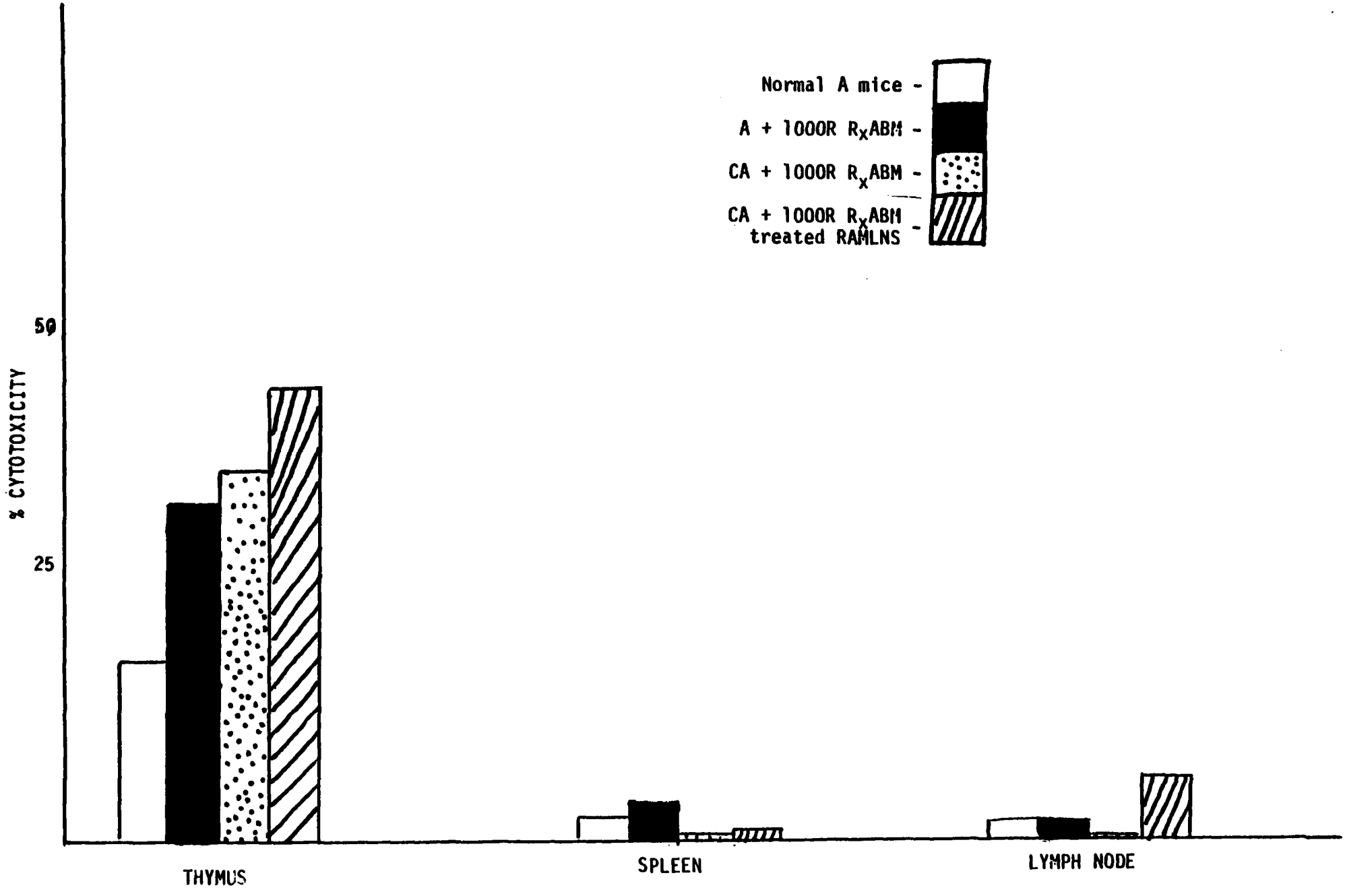


FIGURE 3. LYT 2.2 BEARING CELLS IN:



suppressor T-cells. On the other hand, LYT-1 antisera should kill only helper T-cells. This allows for calculation of cytotoxic/suppressors by subtracting the LYT 1 positive cells from the LYT 2 positive cells. When these calculations are made, again no differences are seen in the ALLOBM and the ALLOBMR$_X$ mice. The results presented here represent a minimum of four mice per point. Similar studies at times after 90 days also reveal no differences in the T-cell subpopulations of ALLOBM and ALLOBMR$_X$ mice. Preliminary results at other times also reveal no differences in the T-cell subpopulations of ALLOBM and ALLOBMR$_X$ mice.

The overall result again argues for the complete elimination of lymphoid elements as the mechanism whereby RAMLNS can, with one initial pretreatment effect the incidence of chronic GVHD mortality. Thus, it appears that if all lymphoid elements, both mature and immature can be removed from a graft not only will acute GVHD be modified but also substantial effects on chronic GVHD can be realized. Thus, the search for agents which can eliminate lymphoid elements from grafts must be continued.

III. The Effect of In Vitro Pretreatment With Monoclonal Antibody Directed Against Various T-Cell Antigens on the Acute Graft-Versus-Host Disease Potential of Mixtures of Murine Spleen and Bone Marrow Cells

As we and others have shown, conventional whole antisera can be very effective agents for the modifications of the GVHD potential of a graft. However, there are problems with the application of conventional whole antisera techniques to clinical bone marrow transplantation. First, every batch of conventional whole antisera will have different properties. Second, the absorption procedures necessary are complex and time consuming. Finally, there is no assay available for directly measuring the effect of an antisera on human pluripotent stem cells. Again, what is needed for in vitro elimination of GVHD potential is an agent which is highly specific for T-cells with no cross-reactivity for stem cells. We feel that monoclonal antibodies directed against T-cell antigens are good candidates for future clinical use in these systems. Monoclonal antibodies would eliminate the problems of non-uniform sera. In addition, absorption would be unnecessary. Finally, monoclonal antibodies are exquisitely specific and therefore should offer no problems of cross-reactivity with stem cells. For these reasons, we evaluated monoclonal antibodies to several T-cell antigens as potential agents for the in vitro pretreatment of murine bone marrow and spleen cell grafts to modify their GVHD potential.

The first step in these studies was to evaluate the in vitro cytotoxicity of the monoclonal antibodies to be studied with our complement source. This was done using a trypan blue exclusion cytotoxicity test. The monoclonal antibodies employed in this study were obtained from New England Nuclear. Table IV shows the in vitro cytotoxicity of anti-THY 1.1 and THY 1.2 monoclonal antibodies on lymphoid cells from A and (C57XA)$_{f1}$ hybrid mice. Both the A strain and the (C57XA)$_{f1}$ hybrid mice are THY 1.2 positive, therefore the anti-THY 1.1 monoclonal antibody serves as a negative control. The anti-THY 1.1 antibody plus complement (C) produced low levels of lysis in all cells tested (8-14%). On the other hand, anti-THY 1.2 plus complement killed 86% of A thymocytes, 82% of (C57XA)$_{f1}$ thymo-cytes and 41% of (C57XA)$_{f1}$ splenocytes at a 1/3000 dilution. The results showed that the anti-THY 1.2 monoclonal was very effective with our source of complement. The other monoclonal antibodies to be used in our experiments were also

TABLE IV

THE IN VITRO CYTOTOXICITY OF MONOCLONAL ANTI THY 1.1 AND THY 1.2 ON LYMPHOID CELLS FROM A AND $(C57XA)_{f1}$ MICE (1/3500 Dilution)

Monoclonal Antibody	% Cytotoxicity To: A Thymus	$(C57XA)_{f1}$ Thymus	$(C57XA)_{f1}$ Spleen
THY 1.1 + C	14%	8%	10%
THY 1.2 + C	86%	82%	41%

tested in this manner and shown to be effective with our complement source.

Table V shows a summary of several experiments measuring the effects of in vitro pretreatment with monoclonal antibodies against T-cell antigens on the acute GVHD potential of murine spleen and bone marrow cells. In these initial experiments, monoclonal antibody to T-cell antigens was added to mixtures of bone marrow and spleen cells in vitro. The mixtures were incubated for 30 minutes at 37°C, then injected into lethally irradiated recipients. In these experiments, no complement was added in vitro since when using whole antisera in this type of experiment, the complement present in the irradiated recipient is sufficient to kill the sensitized cells of the graft. However, as can be seen, the monoclonal antibodies used (THY 1.2, LYT 1.2, and LYT 2.2) were completely ineffective without complement in vitro. All mice receiving bone marrow and spleen cells pretreated with monoclonal antibody in vitro died within 14 days of acute GVHD as did the untreated cell controls. After these initial experiments, all treatments included the addition of complement in vitro. Table VI shows the combined results of several experiments in which a single large inoculum (enough cells for six transplants) was treated in vitro with monoclonal antibody and complement. This treatment technique was employed in all of our previous work with whole antisera. Again, all mice receiving lethal irradiation and untreated cells were dead of acute GVHD by day 14. The mice receiving cells pretreated with THY 1.2 plus complement showed a slight reduction in mortality with 4 of 18 mice surviving more than 14 days. However, the groups receiving mixtures of bone marrow and spleen cells pretreated with LYT 1.2 or LYT 2.2 and complement showed no prolonged survival. These results are somewhat paradoxical with monoclonal antibody plus complement killing T-cells in vitro (Table IV), but with only minimal modification of acute GVHD mortality seen (Table VI). Some possible problems with the use of monoclonal antibody in these experiments may relate to unusually low avidity for the antigen. This can usually be minimized by treatment of the cells with antibody at 4°C, centrifuging and resuspending in media containing complement. This technique was employed in our experiments to no avail. The final dilution of monoclonal antibody used in our initial experiments was 1/3000. The cytotoxic titer of the antibodies used in these experiments was approximately 1/5000; therefore, we were well within the dilution limits of our antibodies. Nevertheless, we have tried higher and lower dilutions of the monoclonal antibody THY 1.2 without increasing the effectiveness of the sera (Table VII). Finally, clumping of lymphoid cells during the treatment period could result in protection of lymphoid cells. Thinking that this phenomenon could result in making our pretreatments with monoclonal antibody less effective, we designed experiments in which smaller aliquots of bone marrow and spleen cell mixtures (enough cells for one transplant) were treated. In these experiments, the cell concentration was lower and the chances of clumping therefore, decreased. The final dilution of monoclonal antibody was 1:1000. The combined results of several experiments done in this manner are presented in Table VIII. Forty mice receiving untreated cells (cell controls) all died by day 14 of acute GVHD. In 24 mice receiving cell mixtures pretreated with monoclonal anti-THY 1.2 and complement acute GVHD mortality was reduced to 75%. In six mice receiving cell mixtures pretreated with monoclonal anti-LYT 1.2 plus complement acute GVHD mortality was reduced to 33%. Finally, in 13 mice receiving cell mixtures pretreated with monoclonal anti-LYT 2.2 plus complement acute GVHD mortality was reduced to 47%. In summary, the technique of using monoclo-

TABLE V

THE EFFECT OF IN VITRO PRETREATMENT WITH MONOCLONAL ANTIBODIES ALONE ON THE ACUTE GVHD POTENTIAL OF MURINE BONE MARROW AND SPLEEN CELLS

Lethally Irradiated Recipient Strain	Number	Treatment	% Mortality 14 Day (Acute GVHD)
$(C57XA)_{f1}$	11	A BM & SPLEEN	100%
$(C57XA)_{f1}$	12	A BM & SPLEEN R_x THY 1.2	100%
$(C57XA)_{f1}$	12	A BM & SPLEEN R_x LYT 1.2	100%
$(C57XA)_{f1}$	12	A BM & SPLEEN R_x LYT 2.2	100%

TABLE VI

THE EFFECT OF IN VITRO PRETREATMENT WITH MONOCLONAL ANTIBODY PLUS COMPLEMENT ON THE ACUTE GVHD POTENTIAL OF MURINE BONE MARROW AND SPLEEN CELLS (1:3000 Dilution)

Lethally Irradiated Recipient Strain	Number	Treatment	% Mortality 21 Day (Acute GVHD)
$(C57XA)_{f1}$	16	A BM & SPLEEN	100%
$(C57XA)_{f1}$	18	A BM & SPLEEN R_X THY 1.2 + C'*	88%
$(C57XA)_{f1}$	12	A BM & SPLEEN R_X LYT 1.2 + C'	100%
$(C57XA)_{f1}$	12	A BM & SPLEEN R_X LYT 2.2 + C'	100%

* Complement

TABLE VII

Dose Response For Monoclonal THY 1.2 Pretreatment

		R_x THY 1.2		
	Untreated Cells	1/1000	1/3000	1/5000
Mortality	6/6	6/6	2/6	5/6

TABLE VIII

THE EFFECT OF IN VITRO PRETREATMENT WITH MONOCLONAL ANTIBODY PLUS COMPLEMENT ON THE ACUTE GVHD POTENTIAL OF MURINE BONE MARROW AND SPLEEN CELLS (1:1000 Dilution)

Lethally Irradiated Recipient Strain	Number	Treatment	% Mortality 21 Day (Acute GVHD)
$(C57XA)_{f1}$	40	A BM & SPLEEN	100%
$(C57XA)_{f1}$	24	A BM & SPLEEN R_X THY 1.2 + C'*	75%
$(C57XA)_{f1}$	6	A BM & SPLEEN R_X LYT 1.2 + C'	33%
$(C57XA)_{f1}$	13	A BM & SPLEEN R_X LYT 2.2 + C'	47%

* Complement

nal antibody to treat small aliquots of cells was the most effective of our monoclonal antibody regimens. However, even in this case, the monoclonal antibodies did not appear to be as effective as conventional whole antisera. We are currently expanding the number of mice in the various treatment groups shown in Table VIII.

The overall success of monoclonal antibodies to T-cell antigens as agents for modifying the acute GVHD potential of murine spleen and bone marrow cell grafts has not been overwhelming in our laboratory. However, we have only investigated a small number of monclonal antibodies thus far. The advantages which monclonal agents hold over conventional whole antisera are many (see above). Therefore, we feel that additional antibodies and different pretreatment approaches with the new and old monoclonal antibodies should be tried before abandoning this approach.

Finally, our studies measuring the effect of pretreatment with monoclonal antibodies to T-cell antigens on the chronic GVHD potential of mouse bone marrow cells are in progress and will be evaluated in the next few months.

KEY FOR ACRONYMS

GVHD - graft-vs-host disease
RAMLNS - rabbit anti-mouse lymph node sera
RAMMLNS - rabbit anti-mouse mesenteric lymph node sera
RAMPLNS - rabbit anti-mouse peripheral lymph node sera
THY 1 - a murine pan T-cell antigen
LYT 1 - a murine T-cell antigen found on helper T-cells
LYT 2 - a murine T-cell antigen found on helper and suppressor-cytotoxic T-cells
LYT 3 - a murine T-cell antigen found on suppressor/cytotoxic T-cells
Ia - a murine antigen found on many lymphoid cells including activated T-cells

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